

allow the identification of hits by DNA sequencing. Alternatively, the sorting module could be used to screen synthetic compounds fixed on beads (e.g. one-bead-one-compound libraries) co-encapsulated in the drops. After the sorting step, beads that mediated the desired effect could be recovered from the drops for a subsequent decoding step (e.g. by mass spectroscopy). Using optical barcodes encoding the compound identity might even allow the decoding step to be performed in real time (without the need for a sorting module). For example, different fluorescence channels could be used for the assay- and label-readout. The optical barcode does not have to be directly linked to the test compound when using droplet-based microfluidics: the label can simply be mixed with the test compound prior to the encapsulation step.

[0234] Aqueous microcompartments can be used as miniaturized vessels for chemical and biological reactions. It has been shown here how this approach can also be utilized for cell-based applications. It has been demonstrated that human cells, and even a multicellular organism (*C. elegans*), can be compartmentalized, and remain fully viable for several days in droplets. The microfluidic platforms described in this set of embodiments allow the encapsulation step at rates of more than 800 per second. As the number of cells per drop follows a Poisson distribution the optional encapsulation of single cells causes the generation of empty drops thus decreasing the resulting encapsulation rate to about 300 per second. It has been demonstrated that post-incubation fluorescence readout of individual compartments at 500 Hz, and further droplet manipulation procedures (such as fusion, splitting and sorting) can be performed at similar rates. Consequently, the throughput of a single integrated droplet-based microfluidic system for cell-based screening could potentially be 500 times higher than conventional robotic microtitre-plate-based HTS technologies which can perform a maximum of ~100,000 assays per day, or $\sim 1 \text{ s}^{-1}$. Using compartments as small as 660 pL, the volume of each assay, and hence the cost of reagents for screening, could be reduced by >1000-fold relative to the smallest assay volumes in microtitre plates (1-2 μL). This may allow many high-throughput biochemical screens to be replaced by more physiologically relevant cell-based assays, including assays using highly valuable cells, e.g. primary human cells, which are arguably the most physiologically relevant model systems, but which generally cannot be obtained on the scale required for HTS. The microfluidic device (FIG. 10A) was fabricated by patterning 75 μm deep channels into poly(dimethylsiloxane) (PDMS) using soft-lithography (Squires and Quake, 2005). The PDMS was activated by incubation for 3 minutes in an oxygen plasma (Plasma Prep 2, Gala Instrument) and bound to a 50 mm \times 75 mm glass slide (Fisher Bioblock). Inlets and outlets were made using 0.75 mm diameter biopsy punches (Harris Uni-Core). The channels were flushed with a commercial surface coating agent (AquaPel, PPG Industries) and subsequently with N₂ prior to use.

[0235] HEK293T cells were grown and encapsulated in DMEM medium (Gibco), Jurkat cells were grown and encapsulated in RPMI medium (Gibco). Both media were supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco). Cells were incubated at 37° C. under a 5% CO₂ atmosphere saturated with water.

[0236] For fluorescence readouts, the lacZ gene was introduced into HEK293T cells by retroviral transduction as described elsewhere (Stitz et al., 2001). In brief, by transfecting HEK293T cells murine leukemia virus-derived particles

(pseudotyped with the G-protein of the vesicular stomatitis virus) were generated that had packaged a vector encoding lacZ. Two days after transfection the particles were harvested from the cell culture supernatants and used for transduction of fresh HEK293T cells during one hour of incubation. Subsequently the cells were cultivated for two weeks before encapsulating them together with 1.7 mM fluorescein di- β -D galactopyranoside (FDG, Euromedex) in drops.

[0237] In brief, surfactants (FIG. 11) were synthesized as follows:

[0238] Carboxy-PFPE. To obtain the ammonium salt of carboxy-PFPE, Krytox FS(L) 2000 (DuPont) was reacted with NH₄OH as described (Johnston et al., 1996).

[0239] DMP-PFPE. Synthesis of the hydrophilic head group dimorpholinophosphate (DMP) was carried out by reaction of PhEtOH (Aldrich), POCl₃ (Fluka) and morpholine (Fluka) with (Et)₃N (Sigma-Aldrich) in THF (Fluka) on ice. Subsequently DMP was coupled to water/cyclohexane/isopropanol extracted Krytox FS(H) 4000 (DuPont) by Friedels-Craft-Acylation.

[0240] PEG-PFPE. Reaction of Krytox FS(H) 4000 (DuPont) with polyethylene glycol (PEG) 900 (Sigma) resulted in a mixture of PEG molecules coupled to either one or two PFPE molecules.

[0241] poly L-Lysine-PFPE. Krytox FS(L) 2000 (DuPont) was reacted with poly L-Lysine (15,000-30,000; Sigma).

[0242] A 100 μL suspension of HEK293T cells (1.5×10^6 cells/ml in fresh media) was seeded on top of a layer of perfluorocarbon oil (FC40, 3M) in the presence (0.5% w/w) and absence of the tested surfactants. After incubation at 37 degrees C. for 48 hours bright light images were taken using a Leica DMIRB microscope.

[0243] Cells were adjusted to a density of 2.5×10^6 cells/ml (determined with a Neubauer counting chamber), stirred at 200 rpm using an 8 mm magnetic stir-bar (Roth) in a 5 ml polyethylene syringe (Fisher Bioblock), and injected via a PTFE tubing (0.56 mm \times 1.07 mm internal/external diameter, Fisher Bioblock) into the microfluidic device (FIG. 10A) using a syringe pump (PhD 2000, Harvard Apparatus) at a flow rate of 1000 microliters/h. The cell suspension was diluted on-chip (see below) by diluting with sterile media (1000 microliters/h if not otherwise stated) and drops were generated by flow-focusing of the resulting stream with perfluorinated oil (FC40, 3M), containing 0.5% (w/w) DMP-PFPE (4000 $\mu\text{L}/\text{h}$). The drop volume was calculated by dividing the flow rate by the drop frequency (determined using a Phantom V4.2 high speed camera). Experimental variations in the drop frequency (at constant flow rates) were defined as the degree of polydispersity in terms of the volume (corresponding to the third power of the polydispersity in terms of the diameter when considering a perfect sphere). For each sample, 500 microliters of the resulting emulsion were collected within a 15 ml centrifuge tube and incubated at 37 degrees C. within a CO₂ incubator (5% CO₂, saturated with H₂O). After incubation, 250 microliters of the emulsion was transferred into a new centrifuge tube and broken by the addition of 15% Emulsion Destabilizer A104 (RainDance Technologies, Guilford, Conn.) and 10 ml of live/dead staining solution (LIVE/DEAD Viability/Cytotoxicity Kit for animal cells, Invitrogen Kit L-3224) and subsequent mixing. After incubation for three minutes (to allow sedimentation of the oil phase) the supernatant was transferred into a 25 cm² tissue culture flask and incubated one hour at room temperature.